# Enhancement of pharmaceuticals treatment on activated sludge process by magnetic activated carbon pretreatment system

Majid Baghdadi*,[1] , Mohammad Javan[1], Tahere Taghizade Firozjaee[2] , Nioushasadat Haji Seyed Javadi[1] , Mahshid Mortazavi[1], Ali Torabian[1]

[1]School of Environment, College of Engineering, University of Tehran, Tehran, Iran.
[2]Faculty of Civil & Architectural Engineering, Department of Civil Engineering, Shahrood University of Technology, Shahrood, Iran.

GRAPHICAL ABSTRACT
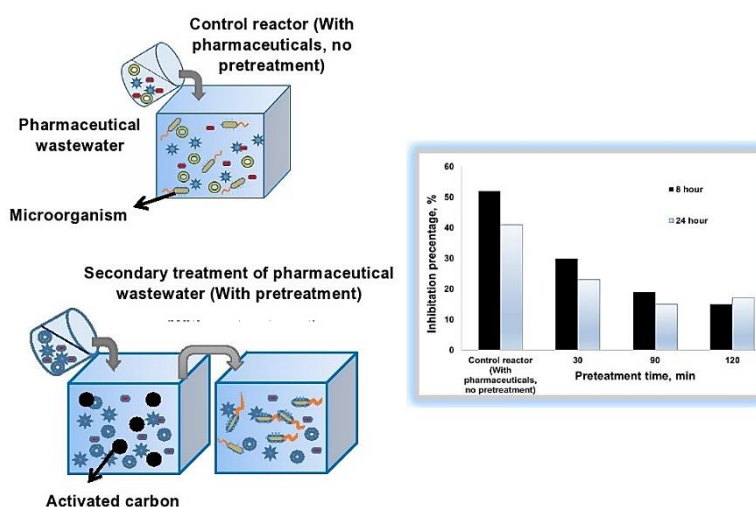
ARTICLE INFO

ABSTRACT

In this study, the effect of antibiotic wastewater containing 20 common pharmaceuticals (14 antibiotics and 6 non-steroidal anti-inflammatory drugs (NSAIDs)) individually as well as their combination was investigated on activated sludge in batch reactors. The chemical oxygen demand (COD), the ammonium concentration, the inhibition rate and toxicity index of COD and ammonium were investigated in wastewater. The inhabitation for COD and ammonium removal was variable for each drug so that the pharmaceuticals are applied simultaneously has such a greater adverse effect on inhibition rate than individual compounds. The pretreatment of wastewaters containing drugs was performed by powdered activated carbon PAC to reduce the adverse effect of these drugs on activated sludge. The appropriate method for separation of PAC from wastewater before introducing to activated sludge process and the optimized adsorption and contact time during the pretreatment process were studied. The pretreatment of pharmaceuticals wastewater with activated carbon improved well COD and NH_4^+ removal to 71 % and 55 %, respectively, that demonstrate the activated carbon can be considered as a suitable pretreatment option for the activated sludge.

1. Introduction

In the past centuries, pharmaceuticals have received global attention for their occurrence and fate in aquatic environments. A Large amount of pharmaceuticals, including antibiotics, are widely used as medications in microbial infection treatment for human beings and animals (Guo et al. 2017). Moreover, some pharmaceutical materials can be applied as fertilizer and as growth promoters for livestock and aquaculture (Kümmerer. 2009). The intensive uses and misuses of pharmaceuticals cause the existence of these compounds in the

---

industrial discharges, hospital sewage effluent, municipal sewage discharge, surface water, groundwater and the sediments (Nayeri et al. 2019; Shokrolahi et al. 2019; Caban and Stepnowski. 2021). During the process of biologically active ingredients production, the amount of antibiotics, which are discharged directly to the environment from the effluent of industrial wastewater treatment, is more than 1 mg/L and, moreover, the concentration of NSAIDs in the influent and effluents of WWTP were reported up to tens of µg/L, and some of them such as naproxen, diclofenac, ibuprofen were found in drinking water (Magureanu et al. 2015). Unfortunately, there are no strict regulations governing the effluent of the pharmaceutical compounds (Larsson. 2014). The continuous presence of pharmaceuticals in the environment has an ecotoxicological Influence on animals (Kümmerer. 2009). A decrease in the reproduction rate of Daphnia Magna and the longevity of nauplii are examples of adverse impacts on the organisms (Wollenberger et al. 2000). The extensive occurrence of antibiotics in the environment is an overriding concern to public health as the antibiotics contribute to the emergence of some resistance genes and bacteria which caused the death of more than 700 000 people per year (Bergeron et al. 2015; Östman et al. 2017). There are a wide variety of ways for pharmaceuticals to enter the environment. Some pharmaceuticals cannot be metabolized completely by the human and animals and some remain intact, which are still active, and then excreted via urine and feces (Hu et al. 2018; Kanakaraju et al. 2018). Besides, as fertilizers contain so many antibiotics, these compounds reach the surface and groundwater through runoff readily (Hirsch et al. 1999). Unfortunately, wastewater treatment plants (WWTPs), as receptive of municipal, industrial, hospital and pharmacies wastewaters, cannot remove pharmaceutical materials completely and consequently these compounds can reach aquatic environments and sediments, which makes the wastewater treatment plants one of the main sources of pharmaceuticals (Kanakaraju and Glass et al. 2018). The conventional WWTPs generally are included a combination of physical and chemical treatments followed by a secondary treatment comprising a biological reactor made by activated sludge (Kim et al. 2005; Rivera-Utrilla et al. 2013). Although the current biological processes are called as low-cost one and can reduce a wide range of pollutants, they are not able to remove micropollutants as well as pharmaceuticals, efficiently (Zhang and Li. 2011; Besha et al. 2017). This inadequate removal efficiency of conventional treatment plants is owning to the complicated molecular structure of pharmaceuticals, their relatively low concentrations in water (Rivera-Utrilla and Sánchez-Polo et al. 2013), and also the molecular properties of these compounds, which determine the biodegradation ability by means of a certain group of microorganisms (Grandclément et al. 2017). For example, trimethoprim and diclofenac are found to be removed by conventional wastewater treatment plants <10 % and 30-40 %, respectively (Hernando et al. 2006). Hernando and his coworkers investigated the removal efficiency of some pharmaceuticals in four sewage treatment plants. According to their results, some pharmaceuticals like tetracycline showed higher removal efficiency (7-73 %) due to its adsorption capacity on particulate matters whereas erythromycin was removed partially (9-19 %) because of its persistency in the environment (Gulkowska et al. 2008). The same result was reported by Bing Li and Tong Zhan who have reported that erythromycin had no removal in the biological treatment process (Li and Zhang. 2010). In another study in Brisbane, Australia the concentration of 28 antibiotics, used for human and veterinary medications, were measured in discharge from activated sludge WWTP, and the results revealed that the antibiotics were still existed in the final effluent (Watkinson et al. 2007). Furthermore, during the process of activated sludge, the pharmaceutical compounds can inhibit the activity of microorganisms which play the primary role of this process (Angeles et al. 2020; Quintelas et al. 2020; Jamialahmadi et al. 2021). According to a study conducted by Dokianakis et al, the adverse effect of seven pharmaceuticals on a community of bacteria, that is nitrite-oxidizing bacteria, was studied. The inhibition of nitrification was observed which can give rise to the existence of nitrite nitrogen in the effluent of wastewater treatment (Dokianakis et al. 2004). Therefore, to improve the elimination of micropollutants and as a result, enhancing the efficiency of biological treatment, a suitable pretreatment process can be a promising option. Some technologies like adsorption, ozonation, and membrane processes are well-suited for the removal of (Knopp et al. 2016) micropollutants (Knopp and Prasse et al. 2016; Benstoem et al. 2017). As it has been noted elsewhere (Delgado et al. 2012)(Grandclément and Seyssiecq et al. 2017), adsorption process in comparison with other processes has a lot more advantages, comprising: more cost-effective, more applicable at low concentrations, easier to operate, more convenient both for continuous and batch

reactors and also their regeneration ability. Among different adsorbents, Powdered Activated Carbon (PAC) is a promising choice that can reduce micropollutants from WWTP (Meinel et al. 2016; Benstoem and Nahrstedt et al. 2017). Since PAC has a smaller particle size in comparison with the other type of activated carbon, granular activated carbon (GAC), it has a higher surface area and as the result, PAC is more efficient for adsorption kinetics (Altmann et al. 2014). Aziz et al investigated two types of sequencing batch reactor (SBR), with and without the addition of PAC, for landfill leachate treating (Aziz et al. 2011). The results showed that PAC improved removal efficiency of COD and NH_3-N. Likewise, according to the study of Kargi et al on biological treatment of pre-treated landfill leachate in the presence of PAC, by adding 2 g/L of PAC, the removal of COD and NH_4-N resulted in 86 % and 26 %, respectively (Kargi and Pamukoglu. 2003). Furthermore, the technology of PAC has also been used in MBR processes for treating wastewater. As an example, Satyawali and his coworkers investigated the treatment of sugarcane wastewater by a membrane bioreactor added with PAC and they obtained higher removal efficiency of COD in the presence of PAC in this process (Satyawali and Balakrishnan. 2009). So, the studies proved that PAC can highly improve the treatment process of wastewater.

In the present study, 20 pharmaceuticals (14 antibiotics and 6 NSAIDs) were chosen because of their intensive uses, their continuous existence in the environment, the harmful effect of NSAIDs on embryos, infants and vulnerable adults, antibiotic resistance bacteria and specifically, their inefficient removal during the conventional WWTPs. The main objective of this research are as follows: (1) the effect of aforementioned compounds individually as well as their combination on activated sludge process; (2) to investigate the impact of activated carbon pretreatment on activated sludge mechanism for treatment of municipal wastewater spiked with mentioned pharmaceuticals; (3) to figure out the appropriate method for separation of PAC from wastewater before introducing to activated sludge process   (4) to optimize the adsorption dose and contact time during the pretreatment process.

2. Material and methods
2.1. Materials

In this study, 6 NSAIDs and 14 antibiotics were investigated. All of these compounds were purchased from Merck (Darmstadt, Germany). Their chemical and physical properties are shown in Table 1. Stock solutions of each compound were prepared in double-distilled water (acid or base) at a concentration of 1000 mg/L. Since piroxicam, cefixime, Trimethoprim, ceftriaxone, mefenamic acid, penicillin, sulfamethoxazole and diclofenac have low solubility in water; these compounds were dissolved into ethanol or acetone. The same amount of ethanol or acetone was added into control samples to consider the impact of these solvents. The ultrasonic bath was used for higher solving of the pharmaceuticals. All the samples in experiments were obtained from the dilution of the stock samples. Activated carbon powder, nitric acid, iron (III) chloride, iron (II) chloride, sulfuric acid were also obtained from Merck (Darmstadt, Germany). During the experiments, double-distilled water was used for preparing solutions. All the wastewater samples were taken daily from the Ikbatan wastewater treatment plant located in Tehran, Iran. The characterization of these samples is presented in Table 2.

2.2. Preparation of magnetic activated carbon

The adsorbent (magnetic activated carbon) is composed of a magnetic core (Fe_3O_4) with a layer of treated activated carbon coated on the core. Magnetic particles were synthesized by chemical co-precipitation method (Li et al. 2007). For this aim, 2.92 g of iron (III) chloride ($FeCl_3.6H_2O$) and 1.05 of iron (II) chloride ($FeCl_2.4H_2O$) were added to distilled water in the presence of nitrogen gas. Then 80 mL of ammonia (NaOH) (65 %) was poured into solution dropwise in 30 minutes. During this process, the mixture was stirred by a mechanical stirrer continuously in the presence of nitrogen gas. In order to prepare carbon active treated by nitric acid, the method presented by Jafari and his coworkers was followed (Jafari Kang et al. 2016). In brief, 40 g of activated carbon was initially added to 200 mL of nitric acid (65 %). This mixture was stirred by a magnetic stirrer for 3 hours at 80 ˚C. Then, nitric acid was separated from the treated activated carbon particles by the use of vacuum pump and filter papers. The nitric acid-treated activated carbon was washed with distilled water and rinsed several times and finally was dried at 50 ˚C in an oven for 24 h. Finally, a 1:4 mixture of nitric acid-treated activated carbon and iron oxide magnetic particles was added into 500 mL of distilled water in the presence of

nitrogen gas. The pH of the solution was adjusted about to 4 and the mixture was stirred for 1 hour at room temperature. The magnetic activated carbon nanoparticles were separated by a magnetic field and dried in the furnace at 50 °C for 12 hours. Then, the nanoparticles were oven-dried at 110 °C for 4 hours. In order to remove iron ions adsorbed on the activated carbon nanoparticles, the final product was washed with HCl (0.2 M) and then with distilled water and finally dried at room temperature.

Table 1. Target compounds and their chemical and physical properties.

Compounds	CAS	Formula	MW	Log K_{ow}	Source
Ciprofloxacin	85721-33-1	$C_{17}H_{18}FN_3O_3$	331.346	0.28	Anti-biotic
Tetracycline	60-54-8	$C_{22}H_{24}N_2O_8$	444.435	-1.37	Anti-biotic
Ofloxacin	82419-36-1	$C_{18}H_{20}FN_3O_4$	361.368	-0.39	Anti-biotic
Sulfamethoxazole	723-46-6	$C_{10}H_{11}N_3O_3S$	253.279	0.89	Anti-biotic
Cefixime	79350-37-1	$C_{16}H_{15}N_5O_7S_2$	453.452	-	Anti-biotic
Ampicillin	69-53-4	$C_6H_{19}N_3O_4S$	349.41	-	Anti-biotic
Trimethoprim	738-70-5	$C_{14}H_{18}N_4O_3$	290.32	0.91	Anti-biotic
Amoxicillin	26787-78-0	$C_{16}H_{19}N_3O_5S$	365.4	0.87	Anti-biotic
Ceftriaxone	73384-59-5	$C_{18}H_{18}N_8O_7S_3$	554.58	-	Anti-biotic
Gentamicin	1403-66-3	$C_{21}H_{43}N_5O_7$	477.596	-1.88	Anti-biotic
Penicillin	113-98-4	$C_9H_{11}N_2O_4S$	243.26		Anti-biotic
Erythromycin	114-07-8	$C_{37}H_{67}NO_{13}$	733.94	3.06	Anti-biotic
Cefalexin	15686-71-2	$C_{16}H_{17}N_3O_4S$	347.39	-	Anti-biotic
Clindamycin	18323-44-9	$C_{18}H_{33}ClN_2O_5S$	424.98	2.16	Anti-biotic
Naproxen	22204-53-1	$C_{14}H_{14}O_3$	230.259	3.18	NSAIDs
Ibuprofen	15687-27-1	$C_{13}H_{18}O_2$	206.29	3.97	NSAIDs
Mefenamic acid	61-68-7	$C_{15}H_{15}NO_2$	241.285	-	NSAIDs
Diclofenac	15307-86-5	$C_{14}H_{11}Cl_2NO_2$	296.148	4.51	NSAIDs
Piroxicam	36322-90-4	$C_{15}H_{13}N_3O_4S$	331.348	-	NSAIDs
Celecoxib	169590-42-5	$C_{17}H_{14}F_3N_3O_2S$	381.373	3.47	NSAIDs

Table 2. The analysis of wastewater samples.

Parameter	Unit	Quantity
COD	mg/L	155
N-NH$_4$	mg/L	24
TN	mg/L	37
TP	mg/L	4.8
pH	-	7.4
TSS	mg/L	110
Temperature	°C	25

2.3. Analytical methods

Chemical oxygen demand (COD) and ammonium concentrations were determined with a UV/VIS spectrophotometer (HACH, DR5000, USA), followed by the standard methods (Baird et al. 2017). According to the mentioned standard method (test number: 5220), for COD determination 2 mL of a sample was added to a COD test kit and stirred for 2 hours at 150 °C. After cooling down at room temperature, the concentration of COD was measured by using DR5000. To determine the amount of ammonium, the pH of the acidified solutions was raised to near 4 to 8 by adding NaOH. Then, 2 mL of the sample was added to a 25 mL volumetric flask and was diluted by distilled water. In the last step, the amount of ammonium was determined based on the Nessler method by using DR5000. Dissolved oxygen was measured by DO meter (Mettler Toledo, USA). The pH of the solutions was measured by pH meter (Metrohm 691, Switzerland). In order to separate fine particles from the liquid phase, cellulose filter papers with the pore size of 25 mm (Sigma-Aldrich, USA) were used.

2.4. Experimental procedures
2.4.1. The effect of individual pharmaceutical on activated sludge process

1 L of activated sludge and 2 L of wastewater were poured in the reactor. 20 mg/L of each pharmaceutical were added and the mixture was aerated by using an aerator pump. During the experiments, the amount of dissolved oxygen was adjusted between 3 to 5 mg/L. At certain intervals, some samples were taken and filtered. In order to prohibit microorganisms' activities in solutions, the mixtures were acidified by adding a certain amount of sulfuric acid. Finally, the amount of ammonium and COD were measured as mentioned before.

2.4.2. The effect of combined pharmaceuticals on activated sludge process

Four experiments with different concentration of pharmaceuticals (0.2, 0.5, 1, and 2 mg/L) were conducted. In each experiment, the specified amount of pharmaceuticals was added to reactors congaing 2 L of wastewater and 1 L of activated sludge. The mixture was aerated

and the amount of oxygen was adjusted between 3 to 5 mg/L. In certain intervals, the samples were taken and filtered.

2.4.3. Two suggested methods for separation of activated carbon from water

In this step, the efficiency of two methods including separation by the magnetic field and coagulation/flocculation were compared for separation of activated carbon at the end of experiments. In these experiments, 1 mg/L of each pharmaceutical was spiked in 2 L of wastewater samples. Wastewater solutions were in contact with activated carbon and magnetic activated carbon for 90 min, in the presence of magnetic stirrer (150 rpm) (Jafari Kang and Baghdadi et al. 2016). For assessing coagulation performance, the jar test apparatus was used and 200 mg/L iron (III) chloride was added to the mixture as a coagulant. The coagulation process was performed in 3 min at 180 rpm and then the particles were flocculated after 15 min stirring at 20 rpm mixing speed.

2.4.4. The effect of magnetic activated carbon on increasing activated sludge process efficiency

In this part, the optimum conditions for activated carbon pretreatment process were examined by two variables including the concentration of adsorbent (1, 3, 5, 6 g/L) and the contact times (30, 90, 120 min). Finally, the mixtures were ready to the transfer to activated sludge process and after sufficient contact time in this reactor, the residual amount of ammonium and COD were determined.

2.5. Introduction of inhibition percentage and Toxicity index parameters

In this research, two parameters of inhibition percentage and toxicity index were introduced in order to examine the adverse effects of pharmaceutical compounds on the activated sludge process. In order to compare the reduction of COD in the control sample and wastewater sample spiked with pharmaceutical compounds, the inhibition parameter was calculated according to Eq. 1, during the first 8 and 24 hours of the experiments.

$$\text{Inhibition percentage} = \frac{\text{rate}_{\text{control sample}} - \text{rate}_{\text{contaminated sample}}}{\text{rate}_{\text{control}}} \times 100 \tag{1}$$

In Eq. 1, rate of control reactor is the reduction of COD or ammonium value in control reactor after 8 or 24 hours of the process and the rate of contaminated samples is the COD or ammonium reduction in reactors spiked with pharmaceutical compounds after 8 or 24 hours of process (Louvet et al. 2010). Furthermore, the kinetic of COD or ammonium removal rate obeyed first-order kinetic equation as follows:

$$\text{Ln} \frac{C_t}{C_0} = -K_t t \tag{2}$$

In Eq. 2, t (s) is the processing time, and C_t and C_0 are the concentration of COD or ammonium in t and at the beginning of the process, respectively. The rate constant of the reaction is presented by K_t (s^{-1}). By equation (2), the rate constant of each experiment is achieved and used for defining toxicity index by the following equation:

$$\text{Toxicity index} = \frac{K_t}{K_0} \tag{3}$$

In Eq. 3, K_0 is the rate constant of the experiment at the beginning of the experiment, and K_t is achieved by equation (2). Toxicity index is an indicator for assessing the toxicity of the reactors and for comparing the toxicity of reactors. According to equation (3), a compound with a higher value of this index shows a lower adverse effect on the activated sludge process. According to our findings, the significant advantage of the toxicity index in comparison with inhibition percentage is that the toxicity index can be used in every desired time after the beginning of the process while inhibition percentage has its most precise result after 8 hours from the beginning of the process.

3. Results and discussion
3.1. Effect of pharmaceuticals compounds individually on COD reduction during the activated sludge process

In order to examine the individual effect of the pharmaceuticals on the process of COD removal, an appropriate amount of each compound was added to wastewater samples to gain a 20 mg/L concentration. Also, a control reactor contained wastewater (without added pharmaceuticals) was prepared in the same experimental conditions to compare the results. COD concentration was measured at 1,2,3,4,5,6,7,8,23 and 24 hours of the experiments. As can be seen in Table 3, the initial COD concentration of pharmaceutical wastewater samples is higher than the COD concentration of the control reactor, which are an obvious result due to the further COD concentrations caused by adding pharmaceuticals. So, it is expected that the control reactor has higher efficiency on the reduction of COD in comparison with reactors containing pharmaceuticals. According to Table 3, the highest percent inhibition (at t=8 hours) was related to mefenamic acid (58 %) and erythromycin (55 %) while the lowest rates were referred to as tetracycline (15 %) and piroxicam (19 %). Likewise, the highest of these findings at t-24 were related to ciprofloxacin (43 %) and erythromycin (41 %) and the lowest ones were referred to diclofenac (11 %) and tetracycline (12 %). Fig.1a to 1d show that the introduction of pharmaceuticals into the reactors caused a great reduction in rate constant of the activated sludge process. It is clear that this decrease is due to slowing down the growth of microorganisms. As shown in Table 3, the percent inhibition for t-24 for all rectors containing pharmaceuticals was much higher than those for t-8. The reason for this phenomenon may be that with the passing time, some microorganisms adapted to new conditions and became more compatible with pharmaceutical compounds, same result has been proved elsewhere (Pasquini et al., 2013). Fig.1a to 1d illustrate that between 2 and 5 hours of the experiments, a small increase in the COD concentration of reactors containing pharmaceuticals was observed. According to the study conducted by Louvet et al, this increase can be attributed to the death of bacteria that released organic material (Louvet et al. 2010).

The toxicity index of each pharmaceutical wastewater reactors is shown in Table 3. As mentioned previously, the inhibition percentage is calculated at t-8 hours, while the toxicity index, resulted from the rate constant of the reaction, is totally independent of a specified time, which is the superiority of this index. Table 3 outlines tetracycline (0.611) and piroxicam (0.527) had the highest toxicity index, respectively and on the other side, erythromycin (0.189) and ibuprofen (0.222) had the lowest value of toxicity index, respectively, for activated sludge process. As can be seen, the maximum percent inhibition belongs to ciprofloxacin, erythromycin, cefalexin, and mefenamic acid. So, it is clear that the findings of these two parameters have the same results, as it was expected .According to research by Louvet and et al, the average inhibition percentage of erythromycin in activated sludge at t^{-1} hours was obtained 79 % Which confirms the result of this test according to the set time period. (Louvet and Giammarino et al. 2010). In another study by Zhang on amoxicillin, this inhibitory effect was observed and its effect on microbial cells was investigated (Zhang and Li. 2011). The inhibition was caused by the death of microbial cells and the release of biomass byproducts in the water .

Table 3. Toxicity index and inhibition percentage of COD and NH_4^+ for reactors containing pharmaceuticals individually.

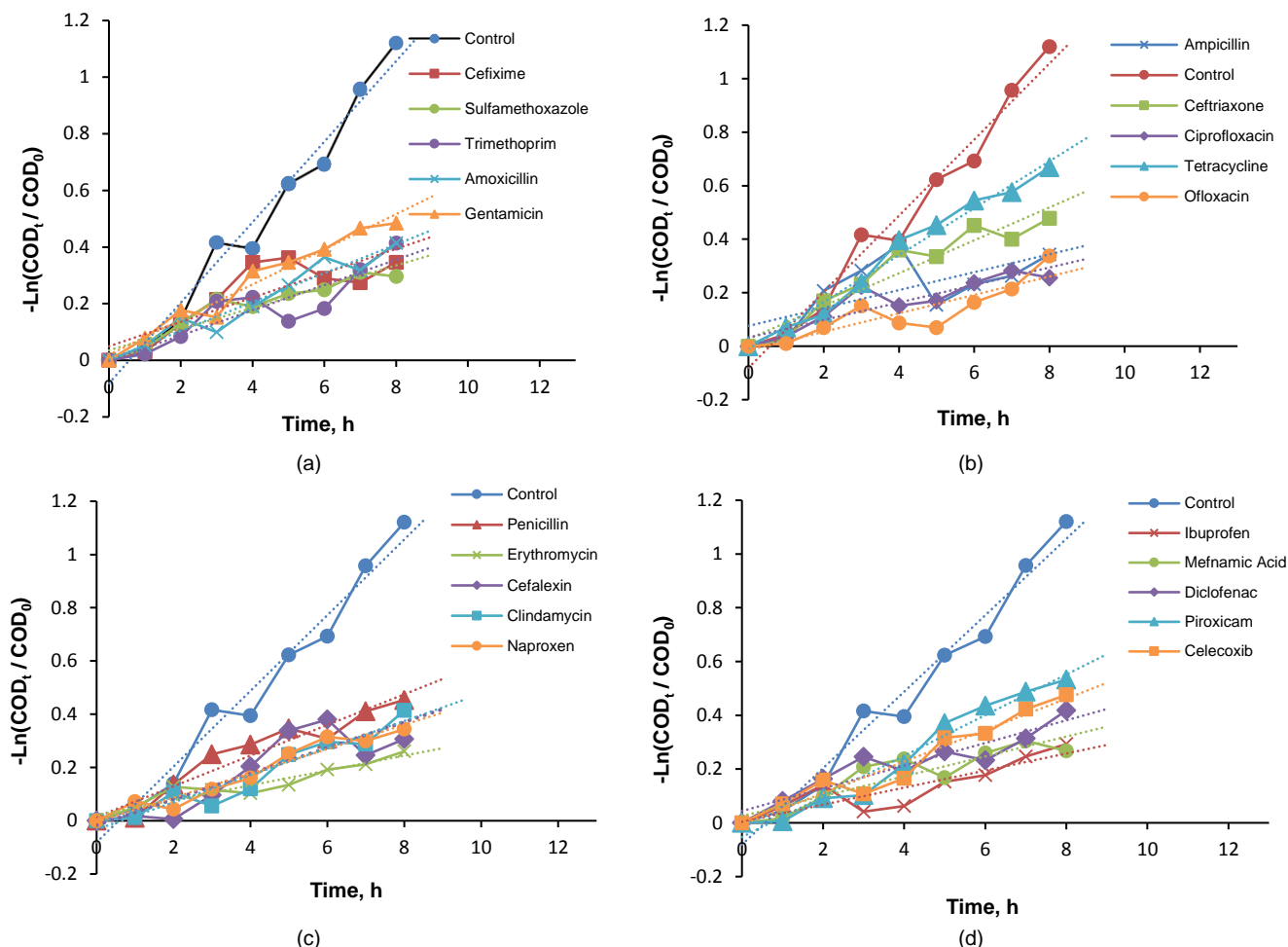| Pharmaceuticals	COD			NH_4^+		
	Percent inhibition t-8h	Percent inhibition t-24h	Toxicity index	Percent inhibition t-8h	Percent inhibition t-24h	Toxicity index
Ciprofloxacin	52	43	0.229	40	22	0.384
Tetracycline	15	12	0.611	21	18	0.506
Ofloxacin	41	28	0.239	53	45	0.336
Sulfamethoxazole	47	38	0.260	41	29	0.322
Cefixime	47	19	0.302	35	30	0.264
Ampicillin	35	29	0.232	32	23	0.508
Trimethoprim	32	27	0.309	34	23	0.536
Amoxicillin	25	22	0.357	30	21	0.456
Ceftriaxone	27	30	0.432	46	41	0.370
Gentamicin	23	26	0.436	16	8	0.625
Penicillin	30	36	0.405	28	24	0.496
Erythromycin	55	41	0.189	44	33	0.333
Cefalexin	51	29	0.337	41	34	0.420
Clindamycin	38	25	0.357	27	12	0.540
Naproxen	41	38	0.316	36	27	0.412
Ibuprofen	46	35	0.222	32	25	0.532
Mefenamic acid	58	32	0.259	50	44	0.282
Diclofenac	29	11	0.296	23	25	0.547
Piroxicam	19	22	0.527	15	11	0.638
Celecoxib	28	32	0.407	18	15	0.601

Fig. 1. Comparison of COD rate constant in reactors containing individual pharmaceutical (20 mg/L) with the COD rate constant in control reactor; (a) For cefixime, sulfamethoxazole, trimethoprim, amoxicillin, gentamicin; (b) For ampicillin, ceftriaxone, ciprofloxacin, tetracycline, ofloxacin; (c) For penicillin, erythromycin, cefalexin, clindamycin, naproxen; (d) For ibuprofen, mefenamic acid, diclofenac, piroxicam, celecoxib

3.2. Effect of each pharmaceutical compounds individually on nitrification

In this stage, in order to investigate the impact of pharmaceuticals on the nitrification process, 20 mg/L of each compound was added to wastewater samples, and the control sample was without the addition of these compounds and examined in the same conditions. The samples were investigated during a 24 h experiment and the sampling were taken at t=1, 2, 3, 4,5,6,7,8,23 and 24 h. Table 3 and Fig. 2a to 2d, show the outcome of these experiments. The results indicate that pharmaceutical wastewater samples had lower nitrification efficiency in comparison with the control reactor. In other words, the pharmaceutical compounds played an inhibitor role in the decomposition of ammonium, held by the microorganism, in the activated sludge process. The inhibition parameter (equation.) was calculated for ammonium reduction at t-8 and 24 hours and the results are depicted in Table 3. At t-8 hours, ofloxacin with 53 % and mefenamic acid with 50 %, showed the highest inhibition percentage, respectively while piroxicam (15 %) and gentamycin (16 %) exhibit the lowest value of inhibition percentage, respectively. Likewise, at t-24 hours, ofloxacin (45 %) and the mefencamic acid (44 %) had the greatest inhibition percentage and on the other side, gentamycin (8 %) and piroxicam (11 %) had the lowest values. It is noted that the ammonium reduction efficiency during the first hour of the reaction in all reactors, even in the control reactor, was too low. because a great deal of shock happened in reactors by introducing new conditions. Furthermore, it was found that the ammonium concentration in wastewater samples contained pharmaceuticals was slightly higher than ammonium concentration in the control reactor. By the results of Fig. 2.a to 2.d, the rate constant of reaction for each reactor can be calculated and be used for analyzing the toxicity index of these reactors, which are summarized in Table 3. According to Table 3, it can be found that piroxicam and gentamycin

had the highest toxicity test with 0.638 and 0.625, respectively while cefixime and mefenamic acid had the lowest value of toxicity index with 0.264 and 0.282, respectively. Like the previous stage, the findings of inhibition percentage and toxicity index for ammonium approve each other.

3.3. The effect of combined pharmaceutics on COD and ammonium reduction during the activated sludge process

In this step, a mixture of all pharmaceutical compounds with a total concentration range from 4 to 40 mg/L plus a control reactor was tested. The inhibition percentage for 8-h and 24-h and the toxicity index were calculated to understand the simultaneous effect of the compounds. These findings are shown in Fig. 3a to 3d and Table 4 depicts the results. In summary, as the total concentration of the pharmaceuticals increased from 4 to 40 mg/L, the inhibition rate for COD at t-8 and t-24 raised from 7 % to 71 % and 5 % to 56 %, respectively, which indicate a chronic inhibition for COD reduction. The same result occurred for ammonium reduction. Likewise, the toxicity index for COD and ammonium removal decreased from 0.77 to 0.08 and 0.76 to 0.14, respectively. The fact that the presence of pharmaceutical compounds can cause the mortality of microorganisms and subsequently a serious problem on their mechanism can be proved by these results. By comparing the outcomes of this section with the results of the previous section, it is understood that when the pharmaceuticals are applied simultaneously had such a greater adverse effect on inhibition rate than individual compound. This consequence is because of the effect of exacerbation phenomenon, which may be due to the molecular interaction of the pharmaceuticals or various half-life of them. These exacerbation effects have been recorded elsewhere previously (Dokianakis and Kornaros et al. 2004).
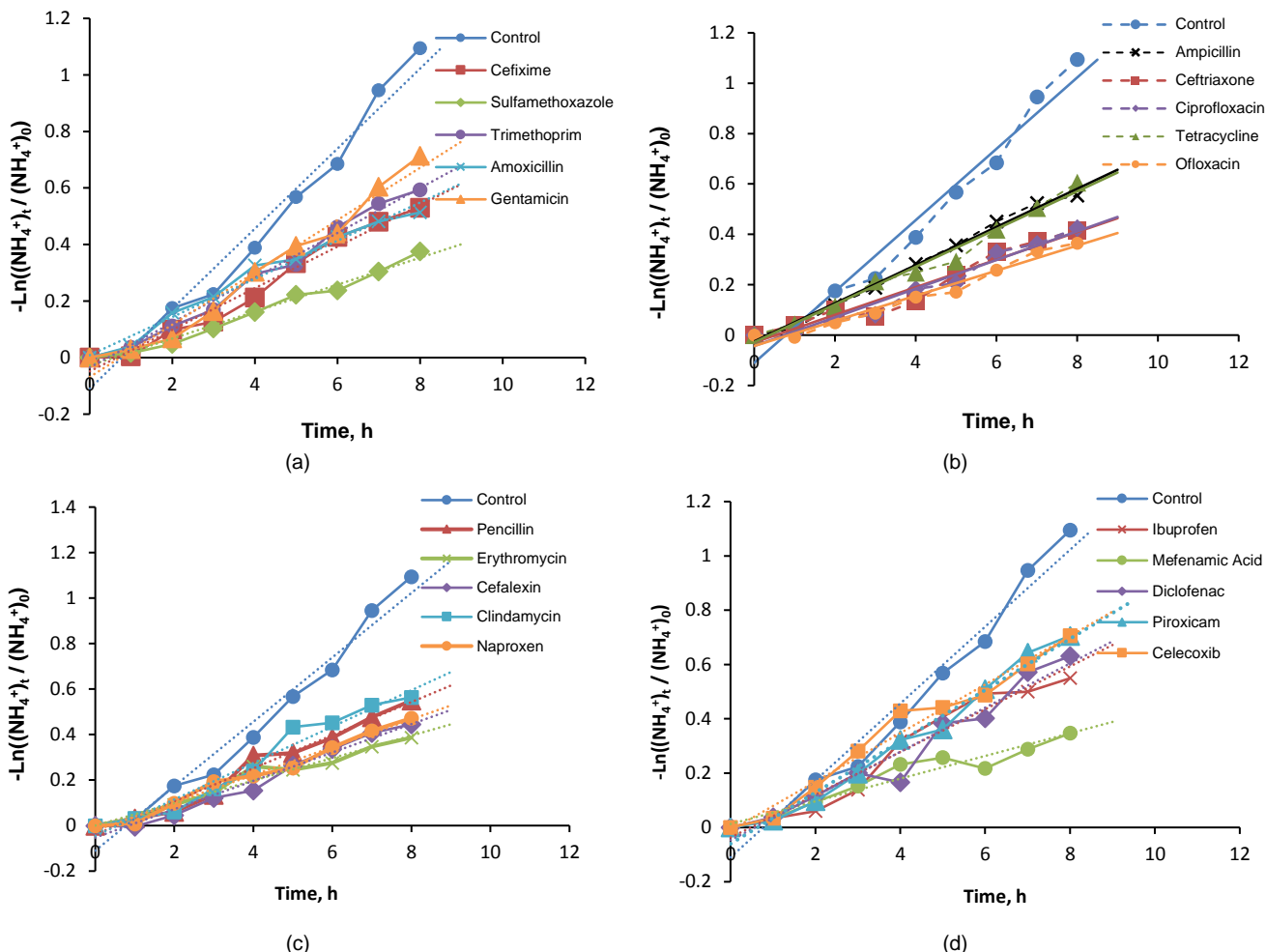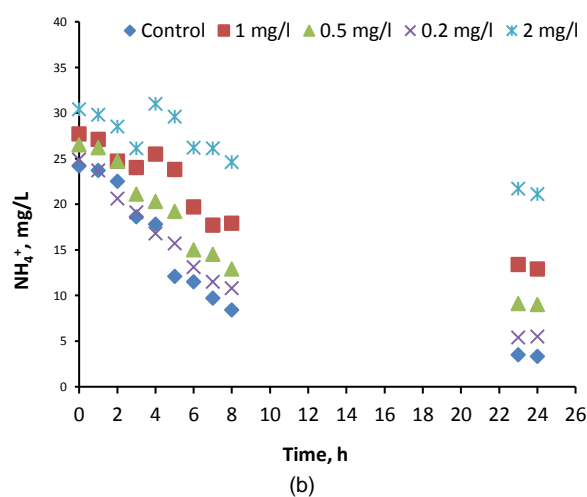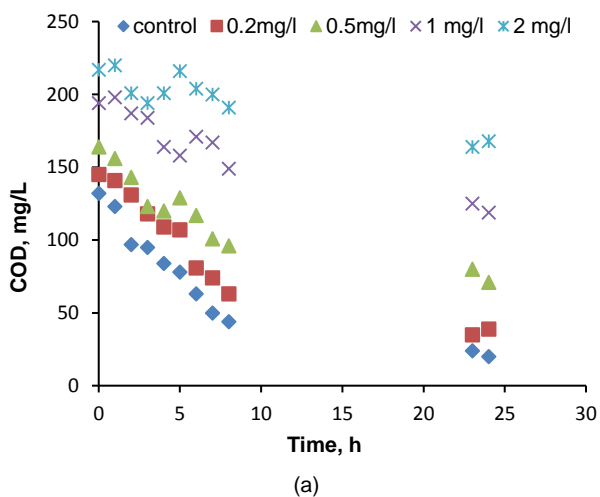
Fig. 2. Comparison of NH$_4^+$ rate constant in reactors containing individual pharmaceutical (20 mg/L) with the NH$_4^+$ rate constant in the control reactor; (a) For cefixime, sulfamethoxazole, trimethoprim, amoxicillin, gentamicin; (b) For ampicillin, ceftriaxone, ciprofloxacin, tetracycline, ofloxacin; (c) For penicillin, erythromycin, cefalexin, clindamycin, naproxen; (d) For ibuprofen, mefenamic acid, diclofenac, piroxicam, celecoxib.

Table 4. Toxicity index and inhibition percentage of COD and NH$_4^+$ for reactors containing combination of pharmaceuticals (20 mg/L in total).

	Parameters	Total concentration of pharmaceuticals, mg/L			
		4	10	20	40
COD	Inhibition percentage, % (t-8h)	7	23	49	71
	Inhibition percentage, % (t-24h)	5	16	33	56
	Toxicity index	0.77	0.46	0.23	0.08
NH$_4^+$	Inhibition percentage, % (t-8h)	11	14	38	62
	Inhibition percentage, % (t-24h)	7	16	29	54
	Toxicity index	0.76	0.65	0.42	0.14

(a)

(b)

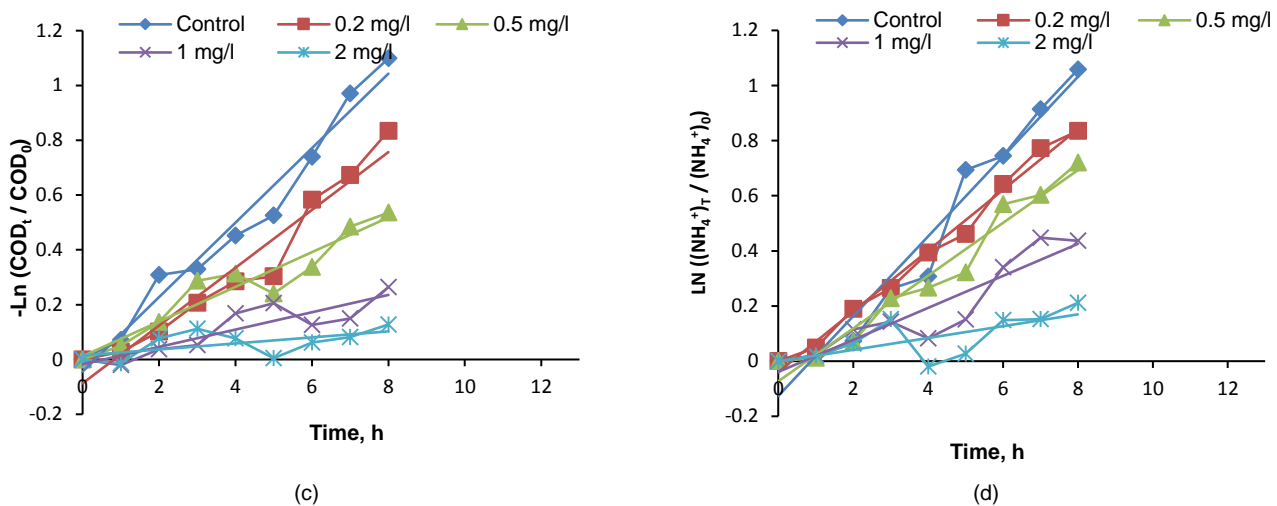(c)   (d)

Fig. 3. (a, b) COD and NH_4^+ concentrations during 24 hours experiments in reactors containing a combination of pharmaceuticals (20 mg/L in total); (c, d) Comparison of COD and NH_4^+ rate constants in reactors containing a combination of pharmaceuticals (20 mg/L in total) with the control reactor.

3.4. Two suggested methods for separation of activated carbon from water mg/L

In this section, an appropriate method has been chosen to prevent the interference of activated carbon during the activated sludge process. Two separation methods including the collection of magnetic activated carbon by the magnetic field and coagulation/flocculation process were applied in the following conditions: 5 g/L of the (magnetic) activated carbon, 20 mg/L of all pharmaceutical (1 mg/L of each), and 90 min contact time.

According to the results, it can be concluded that using the magnet to collect magnetic activated carbon nanoparticles is more efficient than coagulation/flocculation method since as can be seen from Table 5, the inhibition rate resulted by magnetic activated carbon process was 12 while this value was 27 for the coagulation/flocculation method. One of the sensible reasons of this happening is that the amount of C: N: P ratio changes during the coagulation and flocculation process since

phosphorous can be removed in this process, however the optimal C: N: P ratio for aeration tank is 100:10:1 to 100:5:1. Furthermore, it can be concluded that the whole amount of activated carbon was not removed in the coagulation and flocculation process by the applicability of 200 mg/L of iron (III) chloride as the coagulant. The residual amount of activated carbon entering the activated sludge reactor added some non-biodegradable particles to the wastewater; moreover, it made it impossible to make an accurate comparison between the results obtained from control reactors and wastewater reactors spiked with pharmaceuticals. Also, the large amount of sludge produced by the coagulation/flocculation method. Considering the mentioned disadvantage of the coagulation/flocculation process, the magnetic activated carbon separated by a magnetic field as a proper pretreatment method was used for the following experiments. However, may not be completely collected from the system but it will be more effective than other methods.

Table 5. COD concentrations resulted from the two suggested pretreatment methods.

Wastewater sample	COD concentration, mg/L t-0	COD concentration, mg/L t-4h	Inhibition percentage, % t-4h
Control reactor (no pharmaceutical, no pretreatment)	150	91	-
Control reactor (with pharmaceuticals, no pretreatment)	196	160	39
Pharmaceutical wastewater (pre-treated, coagulation separation method)	92	49	27
Pharmaceutical wastewater (pre-treated, magnetic separation method)	139	87	12

3.4. Investigation of optimum conditions for magnetic activated carbon process as a suitable pretreatment process for activated sludge process

To examine the adsorbent doses effect on the COD and ammonium reduction process, five different activated carbon doses (1, 3, 5, 6 g/L) were considered which were added to pharmaceutical wastewater reactors including 20 mg/L of all 20 pharmaceuticals (1 mg/L of each compound). After 90 minutes, the magnetic activated carbon nanoparticles were collected from the solutions by a magnetic field and subsequently the wastewater samples were introduced to the activated sludge reactor which had the same circumstances mentioned before. The control experiment was conducted and all subjects were taken from the same wastewater samples which were provided from the Ikbatan wastewater treatment plant. The results are presented in table 6 and Fig. 4a to 4d. As it can be seen in Fig. 4a and 4b, the COD and NH_4^+ reduction in the presence of even a small amount of PAC are more significant than the COD reduction in the control experiment. As the activated carbon was synthesized at high temperature, its surface

charge is not notable enough to adsorb ions, but according to point of zero charges of magnetic activated carbon in wastewater samples, the activated carbon is negatively charged which causes the more attraction of NH_4^+. The efficiency of the activated sludge process with a magnetic activated carbon pretreatment process (PAC concentration at 6 mg/L) was 70 % higher at t=8 hour than the control experiment with no PAC. Likewise, in the case of NH_4^+, the efficiency of the activated sludge in the presence of activated carbon (6 g/L) is 56 % greater than the control experiments' efficiency. According to Fig 4c to 4d and Table 6, as the PAC doses increased, a great increase in the rate constant and consequently in toxicity index of both COD and NH_4^+ occurred which brings up the point that the larger amount of the adsorbent is more effective in the reduction of COD and NH_4^+.Similar results have been reported in other studies (Park et al. 2003; Hu et al. 2015). The results shown in Table 6 revealed that by increasing the amount of PAC to 5 g/L, inhibition percentage decreased remarkably; however, the percent inhibition did not decrease notably as the amount of magnetic activated carbon exceeded 5 mg/L. Hence, it can be concluded that the optimum amount of magnetic activated carbon is 5 mg/L.
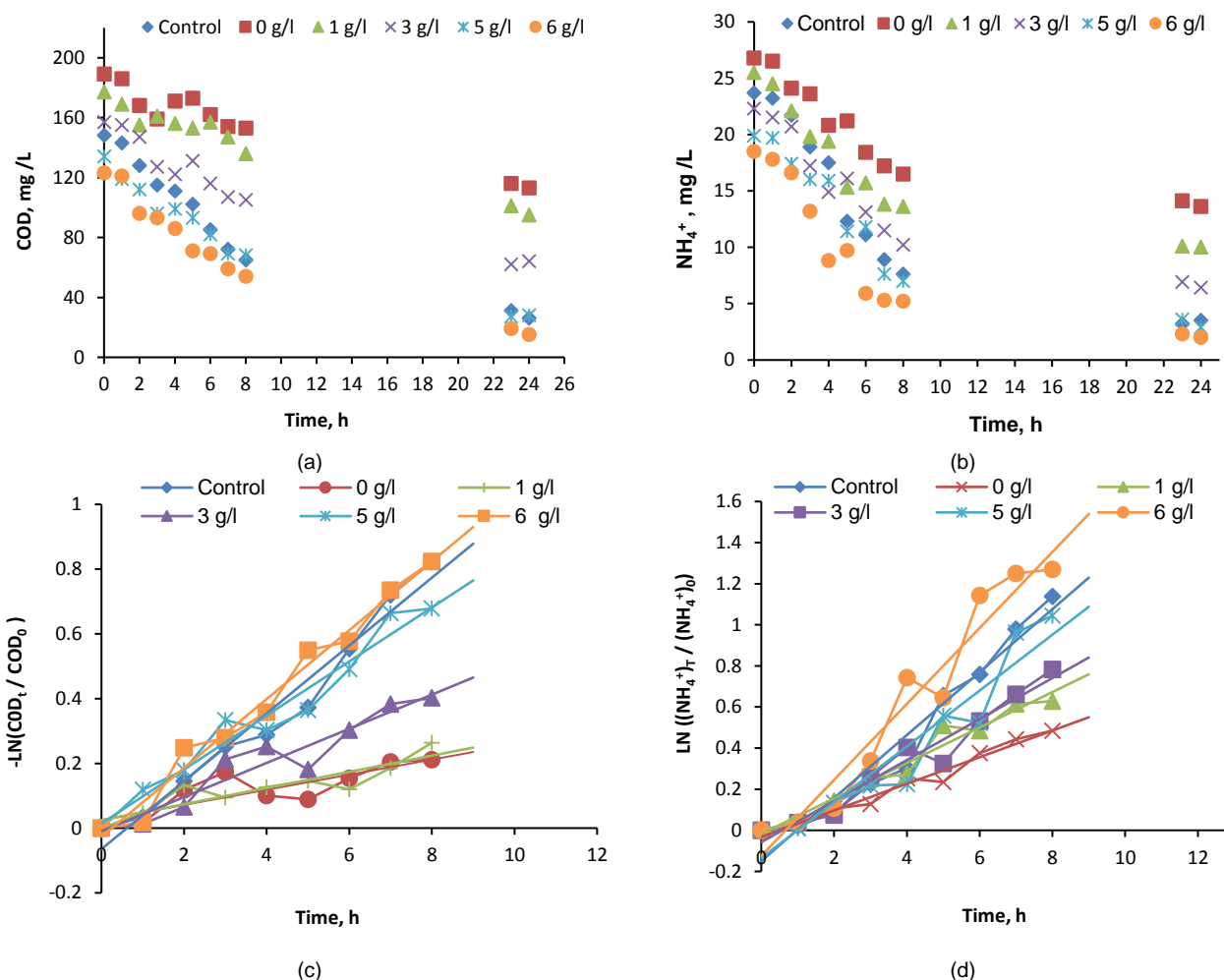
156

(a)

(b)

(c)

(d)

Fig. 4. (a, b) COD and NH_4^+ concentrations during 24 hours experiments in reactors containing combination of pharmaceuticals (20 mg/Lin total) at different PAC concentrations (0, 1, 3, 5 and 6 g/L); (c, d) Comparison of COD and NH_4^+ rate constants in reactors containing combination of pharmaceuticals (20 mg/L in total) at different PAC concentrations (0, 1, 3, 5 and 6 g/L) with the control reactor.

Table 6. COD and NH_4^+ inhibition percentage, % and toxicity index at different PAC concentrations.

Parameter	Parameter	PAC concentration, g/L				
		0	1	3	5	6
COD	Inhibition percentage, % (t-8h)	57	51	38	21	17
	Inhibition percentage, % (t-24h)	38	33	24	14	11
	Toxicity index	0.22	0.24	0.50	0.91	1.01
NH_4^+	Inhibition percentage, % (t-8h)	38	32	25	20	17
	Inhibition percentage, % (t-24h)	35	30	21	16	18
	Toxicity index	0.41	0.56	0.64	0.92	1.07

Furthermore, to figure out the efficient contact time during the pretreatment process, 2 L of the pharmaceutical wastewater, which contains 20 mg/L of all aforementioned pharmaceuticals, was contacted with magnetic activated carbon (5 g/L) for 30, 90, and 120 minutes. At the end of the experiments, magnetic activated carbons were collected by a magnetic field. Subsequently, the solutions were poured into three reactors and entered the activated sludge process. Two control reactors were run, one of them contained pure wastewater and the other consisted of a mixture of all 20 pharmaceuticals (1 mg/L of each compound). Based on the results shown in Table 7 and Fig. 5a

to 5d, by increasing the contact time, a considerable decrease was observed in the rate constant and percent inhibition for NH_4^+ and COD. However, when the contact time exceeded 90 min, no remarkable change was seen for this parameter. For example, after 120 min activated carbon pretreatment, the percent inhibition of COD and NH_4^+ for t-8 hours was just 4 % and 1 %, respectively, lower than these two values which resulted from 90 min activated carbon pretreatment. The same finding was obtained for the toxicity index value in Table 7. Therefore, it can be concluded that the optimum contact time for the activated carbon pretreatment process is 90 min.

Table 7. COD and NH_4^+ inhibition percentage and toxicity index at different pretreatment contact times.

Parameter	Parameter	Pretreatment contact time, min			
		0*	30	90	120
COD	Inhibition percentage, % (t-8h)	52	30	19	15
	Inhibition percentage, % (t-24h)	41	23	15	17
	Toxicity index	0.25	0.60	0.84	0.99
NH_4^+	Inhibition percentage, % (t-8h)	38	32	22	18
	Inhibition percentage, % (t-24h)	35	25	18	17
	Toxicity index	0.36	0.56	0.95	1.07

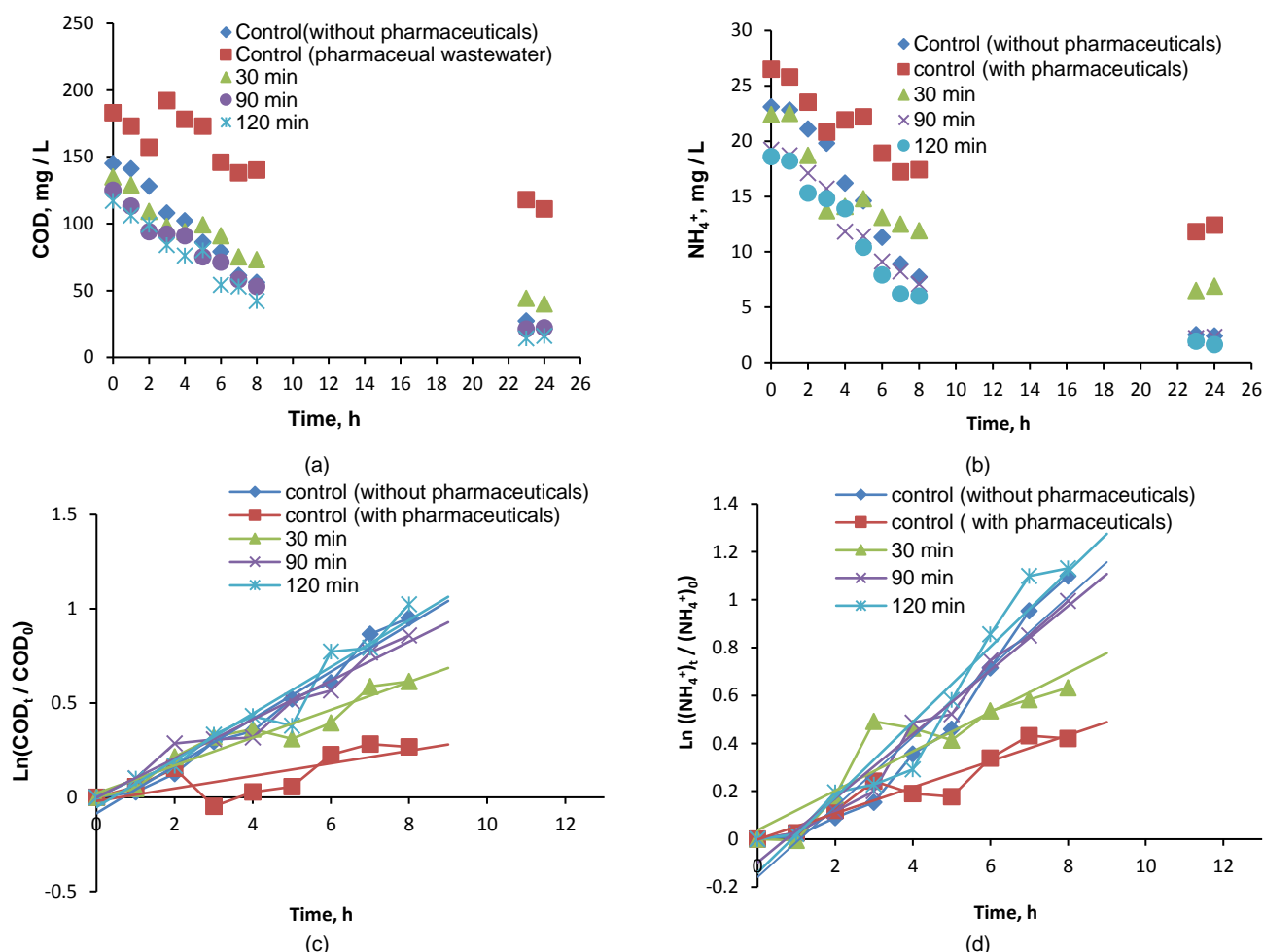*Control reactor containing the combination of pharmaceuticals (20 mg L^{-1} in total)

Fig. 5. (a, b) COD and NH_4^+ concentrations during 24 hours experiments in reactors containing combination of pharmaceuticals (20 mg/L in total) with different pretreatment contact times (30, 90 and 120 min) and control reactors; (c, d) COD and NH_4^+ rate constants in reactors containing combination of pharmaceuticals (20 mg/L in total) with different pretreatment contact times (30, 90 and 120 min) and control reactors.

4. Conclusions

All of the 20 pharmaceuticals had a negative impact on the activated sludge process both for COD and nitrate reduction. However, this reduction differs from one compound to another; mefenamic acid and erythromycin with 58 % and 55 % had the highest inhibition rate of COD removal while the lowest value for this parameter referred to tetracycline (15 %) and piroxicam (19 %). The same result was obtained from the toxicity index of COD which showed tetracycline (0.611) and piroxicam (0.527) with the most toxicity index while erythromycin and ibuprofen had the lowest toxicity index, with 0.189 and 0.222, respectively. In terms of NH_4^+, ofloxacin and mefenamic acid with 53 % and 50 % had the greatest amount of inhibition and on the other side, piroxicam and gentamycin with 15 % and 16 % respectively. Likewise, regarding the toxicity index, piroxicam and gentamycin with 0.638 and 0.625, respectively showed the greatest value while cefixime and mefenamic acid with 0.264 and 0.282 exhibited the lowest value. The presence of all 20 pharmaceuticals in wastewater (20 mg/L) played the more extensive inhibitory role for COD and ammonium reduction in comparison with the presence of almost each compound separately (20 mg/L), which is can be due to the negative interaction of pharmaceuticals on each other and further researches are needed to disclose this subject. The addition of activated carbon in the form of powder as a pretreatment process exhibited a significant effect on COD and NH_4^+ removal; this process improved COD and NH_4^+ removal to 71 % and 55 %, respectively. The optimum concentration of PAC was 5000 mg/L which sufficiently decreased the inhibition rate for COD and NH_4^+ in wastewater added with all pharmaceuticals (20 mg/L in total) to 14 % and 16 %, respectively. Bedsides, the optimum contact time during the pretreatment process was around 90 min. The results show that pharmaceuticals cause an extensive inhibitory effect on COD and ammonium removal in the activated sludge process also activated carbon is an appropriate pretreatment for the activated sludge.

References

Altmann J., Ruhl A.S., Zietzschmann F., Jekel M., Direct comparison of ozonation and adsorption onto powdered activated carbon for micropollutant removal in advanced wastewater treatment, Water Research 55 (2014) 185-193.

Angeles L.F., Mullen R.A., Huang I.J., Wilson C., Khunjar W., Sirotkin H.I., McElroy A., E.Aga D.S., Assessing pharmaceutical removal and reduction in toxicity provided by advanced wastewater treatment systems, Environmental Science: Water Research & Technology 6 (2020) 62-77.

Aziz S.Q., Aziz H.A., Yusoff M.S., Bashir M.J., Landfill leachate treatment using powdered activated carbon augmented sequencing batch reactor (SBR) process: Optimization by response surface methodology, Journal of Hazardous Materials 189 (2011) 404-413.

Baird R.B., Eaton A.D., Rice E.W., Bridgewater L., Standard methods for the examination of water and wastewater, American Public Health Association Washington, DC, (2017).

Benstoem F., Nahrstedt A., Boehler M., Knopp G., Montag D., Siegrist H.Pinnekamp J., Performance of granular activated carbon to remove micropollutants from municipal wastewater—A meta-analysis of pilot- and large-scale studies, Chemosphere 185 (2017) 105-118.

Bergeron S., Boopathy R., Nathaniel R., Corbin A., LaFleur G., Presence of antibiotic resistant bacteria and antibiotic resistance

genes in raw source water and treated drinking water, International Biodeterioration & Biodegradation 102 (2015) 370-374.

Besha A.T., Gebreyohannes A.Y., Tufa R.A., Bekele D.N., Curcio E., Giorno L., Removal of emerging micropollutants by activated sludge process and membrane bioreactors and the effects of micropollutants on membrane fouling: A review, Journal of Environmental Chemical Engineering 5 (2017) 2395-2414.

Caban M., Stepnowski P., How to decrease pharmaceuticals in the environment? A review, Environmental Chemistry Letters (2021) 1-24.

Dokianakis S., Kornaros M., Lyberatos G., On the effect of pharmaceuticals on bacterial nitrite oxidation, Water Science and Technology 50 (2004) 341-346.

Grandclément C., Seyssiecq I., Piram A., Wong-Wah-Chung P., Vanot G., Tiliacos N., Roche N.Doumenq P., From the conventional biological wastewater treatment to hybrid processes, the evaluation of organic micropollutant removal: a review, Water Research 111 (2017) 297-317.

Gulkowska A., Leung H. W., So M. K., Taniyasu S., Yamashita N., Yeung L. W., Richardson B. J., Lei A., Giesy J. P.Lam P. K., Removal of antibiotics from wastewater by sewage treatment facilities in Hong Kong and Shenzhen, China, Water Research 42 (2008) 395-403.

Guo J., Li J., Chen H., Bond P. L.Yuan Z., Metagenomic analysis reveals wastewater treatment plants as hotspots of antibiotic resistance genes and mobile genetic elements, Water Research 123 (2017) 468-478.

Hernando M.D., Mezcua M., Fernández-Alba A.R., Barceló D., Environmental risk assessment of pharmaceutical residues in wastewater effluents, surface waters and sediments, Talanta 69 (2006) 334-342.

Hirsch R., Ternes T., Haberer K., Kratz K.L., Occurrence of antibiotics in the aquatic environment, Science of The Total Environment 225 (1999) 109-118.

Hu J., Zhou J., Zhou S., Wu P., Tsang Y.F., Occurrence and fate of antibiotics in a wastewater treatment plant and their biological effects on receiving waters in Guizhou, Process Safety and Environmental Protection 113 (2018) 483-490.

Hu Q.Y., Li M., Wang C., Ji M., Influence of powdered activated carbon addition on water quality, sludge properties, and microbial characteristics in the biological treatment of commingled industrial wastewater, Journal of Hazardous Materials 295 (2015) 1-8.

Jafari Kang A., Baghdadi M., Pardakhti A., Removal of cadmium and lead from aqueous solutions by magnetic acid-treated activated carbon nanocomposite, Desalination and Water Treatment 57 (2016) 18782-18798.

Jamialahmadi N., Rahimi S., Esmaeili A., Hospital wastewater in Iran: a systematic review and challenges for proper management during coronavirus disease (2019) pandemic, Journal of Applied Research in Water and Wastewater 8 (2021) 59-65.

Kanakaraju D., Glass B.D., Oelgemöller M., Advanced oxidation process-mediated removal of pharmaceuticals from water: a review, Journal of Environmental Management 219 (2018) 189-207.

Kargi F.Pamukoglu M.Y., Powdered activated carbon added biological treatment of pre-treated landfill leachate in a fed-batch reactor, Biotechnology Letters 25 (2003) 695-699.

Kim S., Eichhorn P., Jensen J. N., Weber A.S., Aga D.S., Removal of antibiotics in wastewater: effect of hydraulic and solid retention times on the fate of tetracycline in the activated sludge process, Environmental Science & Technology 39 (2005) 5816-5823.

Knopp G., Prasse C., Ternes T.A., Cornel P., Elimination of micropollutants and transformation products from a wastewater treatment plant effluent through pilot scale ozonation followed by

various activated carbon and biological filters, Water Research 100 (2016) 580-592.

Kümmerer K., Antibiotics in the aquatic environment–a review–part I, Chemosphere 75 (2009) 417-434.

Kümmerer K., Antibiotics in the aquatic environment–a review–part II, Chemosphere 75 (2009) 435-441.

Larsson D.J., Antibiotics in the environment, Upsala Journal of Medical Sciences 119 (2014) 108-112.

Li B., Zhang T., Biodegradation and adsorption of antibiotics in the activated sludge process, Environmental Science & Technology 44 (2010) 3468-3473.

Li Y., Liu Y., Tang J., Lin H., Yao N., Shen X., Deng C., Yang P.Zhang X., $Fe_3O_4@ Al_2O_3$ magnetic core–shell microspheres for rapid and highly specific capture of phosphopeptides with mass spectrometry analysis, Journal of Chromatography A 1172 (2007) 57-71.

Louvet J.N., Giammarino C., Potier O.Pons M.N., Adverse effects of erythromycin on the structure and chemistry of activated sludge, Environmental Pollution 158 (2010) 688-693.

Magureanu M., Mandache N. B.Parvulescu V.I., Degradation of pharmaceutical compounds in water by non-thermal plasma treatment, Water Research 81 (2015) 124-136.

Meinel F., Zietzschmann F., Ruhl A., Sperlich A.Jekel M., The benefits of powdered activated carbon recirculation for micropollutant removal in advanced wastewater treatment, Water Research 91 (2016) 97-103.

Nayeri D., Mousavi S.A., Mehrabi A., Oxytetracycline removal from aqueous solutions using activated carbon prepared from corn stalks, Journal of Applied Research in Water and Wastewater 6 (2019) 67-72.

Östman M., Lindberg R.H., Fick J., Björn E.Tysklind M., Screening of biocides, metals and antibiotics in Swedish sewage sludge and wastewater, Water Research 115 (2017) 318-328.

Park S.J., Oh J.W., Yoon T.I., The role of powdered zeolite and activated carbon carriers on nitrification in activated sludge with inhibitory materials, Process Biochemistry 39 (2003) 211-219.

Quintelas C., Mesquita D.P., Torres A.M., Costa I.Ferreira E.C., Degradation of widespread pharmaceuticals by activated sludge: Kinetic study, toxicity assessment, and comparison with adsorption processes, Journal of Water Process Engineering 33 (2020) 101061.

Rivera-Utrilla J., Sánchez-Polo M., Ferro-García M.Á., Prados-Joya G.Ocampo-Pérez R., Pharmaceuticals as emerging contaminants and their removal from water, A review, Chemosphere 93 (2013) 1268-1287.

Satyawali Y., Balakrishnan M., Performance enhancement with powdered activated carbon (PAC) addition in a membrane bioreactor (MBR) treating distillery effluent, Journal of Hazardous Materials 170 (2009) 457-465.

Shokrolahi S., Farhadian M.Davari N., Degradation of Enrofloxacin antibiotic in contaminated water by ZnO/Fe2O3/Zeolite nanophotocatalyst, Journal of Applied Research in Water and Wastewater 6 (2019) 150-155.

Watkinson A., Murby E.Costanzo S., Removal of antibiotics in conventional and advanced wastewater treatment: implications for environmental discharge and wastewater recycling, Water Research 41 (2007) 4164-4176.

Wollenberger L., Halling-Sørensen B, Kusk K.O., Acute and chronic toxicity of veterinary antibiotics to Daphnia magna, Chemosphere 40 (2000) 723-730.

Zhang T., Li B., Occurrence, transformation, and fate of antibiotics in municipal wastewater treatment plants, Critical Reviews in Environmental Science and Technology 41 (2011) 951-998.